Individual severity of dietary obesity in unselected Wistar rats: relationship with hyperphagia

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Harrold, Joanne A., Peter S. Widdowson, John C. Clapham, and Gareth Williams. Individual severity of dietary obesity in unselected Wistar rats: relationship with hyperphagia. Am J Physiol Endocrinol Metab 279: E340–E347, 2000.—We investigated the relative importance of overeating, thermogenesis, and uncoupling protein (UCP) expression in determining the severity of obesity in male Wistar rats fed a highly palatable diet. After 2 wk of feeding, body weight did not differ significantly from controls (248 ± 4 vs. 229 ± 3 g; P > 0.3), but rectal temperature, brown adipose tissue (BAT) mass, UCP3 expression in gastrocnemius muscle, and UCP2 expression in white adipose tissue (WAT) were all elevated in diet-fed animals. In a further study, rats fed a palatable diet for 8 wk exhibited higher energy intake and rectal temperature than controls. Dietary-obese rats were divided into high (427–490 g; n = 8) and low (313–410 g; n = 8) weight gainers. The high gainers ate significantly more than the low gainers, and energy intake and rectal temperature were significantly increased above lean controls in all diet-fed animals, whereas UCPs in WAT and BAT did not differ significantly from controls. Whereas rats fed palatable food exhibited a thermogenic response, there was no significant difference in core temperature between high and low gain groups (37.5 ± 0.1 vs. 37.6 ± 0.1°C; P > 0.5). We conclude that a higher energy intake is the critical factor determining susceptibility to dietary obesity in unselected Wistar rats.

food intake; uncoupling proteins; thermogenesis; palatable diet

Dietary obesity, induced by exposure to highly palatable food, is the rodent model that most closely resembles human obesity. The severity of obesity varies considerably between individuals, and it is not clear whether this is attributable to differences in appetite for palatable food or to the metabolic adaptations that tend to limit excessive weight gain. An important adaptive mechanism is an increase in metabolic rate, which in rodents has been attributed largely to enhanced energy expenditure by brown adipose tissue (BAT), the principal thermogenic tissue whose activity can be varied according to need (11).

Uncoupling protein 1 (UCP1), expressed solely in BAT (15), is firmly implicated in modulating energy disposal during states of altered energy balance. UCP1 is an integral protein of the inner mitochondrial membrane, which functions to translocate protons into the mitochondrial matrix so that the oxidation of free fatty acids results in heat production rather than ATP synthesis (19).

Recently, two genes with high sequence homology to UCP1 were identified and named UCP2 (5) and UCP3 (1, 28). They display tissue-selective distributions: UCP3 is largely confined to BAT and skeletal muscle, whereas UCP2 occurs much more widely in rodent and human tissues (5). Because of their structural similarity to UCP1, it has been suggested that UCP2 and UCP3 also function to facilitate proton transport, and there is in vitro evidence to support this view (5); however, this proposed thermogenic role remains circumstantial at present. Alternative suggested functions include involvement in the utilization of free fatty acids as fuel substrates, for example, during prolonged fasting when stored fats are mobilized to meet ongoing energy needs (29).

The roles of the various UCPs in regulating energy expenditure and body adiposity in rodents remain controversial. Heat production in BAT has generally been attributed to UCP1 and is thought to be important in determining whole body energy expenditure. BAT ablation by transgenic expression of diphtheria toxin leads to obesity (16); interestingly, mice that are deficient in UCP1 (UCP1 knockouts) do not become obese when fed either normal chow or a high-calorie diet, but they are cold intolerant (4). This suggests that UCP1 induction in BAT is important for generating heat, primarily during periods of cold exposure, and that other thermogenic proteins in BAT may be more important in determining body adiposity, e.g., UCP2.
whose expression is increased in the BAT of UCP1 knockout mice (4).

The role of UCP1 in the increased thermogenesis of dietary obesity is also controversial. Elevated BAT UCP1 levels have been reported in studies of young rats fed a cafeteria diet (22), although we found no changes in UCP1 mRNA concentrations in older dietary-obese rats that had raised body temperature (31). This discrepancy may be explained by attenuation of obesity-related thermogenesis in older rats (21) or alternatively by differences in dietary composition; for example, enrichment with polyunsaturated fatty acids has been shown to stimulate BAT thermogenic activity, measured by guanosine diphosphate (GDP) binding, without inducing changes in the UCP content (18).

Recent studies have suggested that UCP2 expression in adipose tissue may determine the degree of adiposity in different strains of mice fed a high-fat diet (25). Obesity-resistant (A/J) mice responded to high-fat feeding with significantly elevated UCP2 expression in white adipose tissue (WAT), whereas obesity-prone (C57BL/6) mice fed the same diet did not show any changes in WAT UCP2 expression despite increased fat mass (25). BAT UCP1 expression increased significantly in both strains of mice, although to a greater extent in the obesity-resistant strain, which also showed a significant rise in core temperature (24). Food intake was not different between the two strains of mice, suggesting that, at least in these mice, the induction of UCP2 expression in WAT and UCP1 in BAT was important in limiting the severity of adiposity in high-fat feeding.

In these mice (25), the different responses of UCP2 induction and the propensity to weight gain are determined by the strain. The importance of the UCPs in individual susceptibility to dietary-induced obesity, which is not genetically determined, has not been systematically studied. Here, we examined UCP expression in interscapular BAT, WAT, and gastrocnemius muscle, along with measurements of food intake and several indicators of thermogenic activity (core body temperature and purine nucleotide binding), in genetically uniform Wistar rats exposed to a highly palatable diet. We previously concluded that increased UCP2 expression in WAT showed a significant rise in core temperature (24). Food intake was not different between the two strains of mice, suggesting that, at least in these mice, the induction of UCP2 expression in WAT and UCP1 in BAT was important in limiting the severity of adiposity during high-fat feeding.

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**METHODS AND MATERIALS**

**Animals**

Eight-week-old male Wistar rats (150 g), obtained from Liverpool University breeding stock, were randomized and assigned to control (\(n = 25\)) or to palatable diet-fed groups (\(n = 34\)). All had free access to water and were housed in groups of two or three under controlled environmental conditions (19–22°C, 30–40% humidity) and a 12:12-h light-dark cycle (lights on at 0700). Control rats were fed a standard laboratory pelleted diet (CRM Biosure, Cambridge, UK), whereas all others had free access to a highly palatable high-energy diet consisting of 33% ground pellet diet, 33% Nestlé condensed milk, 7% sucrose, and 27% water, as previously described (30). Body weights were recorded every week, and the animals were inspected daily.

After 2 wk, rectal temperatures in eight control and eight palatable diet-fed rats were measured at the beginning of the light cycle with a digital thermometer inserted ~5 cm and held until a steady peak temperature was recorded (~15 s). Animals had been previously habituated to handling, ensuring that minimal restraint was required during this procedure, thus avoiding any artificial increases in body temperature. They were then killed by CO\(_2\) inhalation, and blood was removed by cardiac puncture into cold heparinized tubes. The plasma was immediately separated by centrifugation before being frozen for later measurement of blood analytes. The gastrocnemius muscle, interscapular BAT, and epididymal and perirenal fat pads were dissected, cleaned of any surrounding fat and muscle, weighed, and snap-frozen in liquid N\(_2\). Tissues were stored at −40°C until used for Northern blotting (~1 mo after the animals were killed) or free fatty acid and triglyceride measurements.

The remaining rats were maintained on their respective diets for a total of 8 wk. For the final 2 wk, they were housed singly, and individual food intake was measured over 5 days. During the final week, rectal temperatures were again assessed at the onset of the light phase. Animals were killed in a temperature-controlled room maintained at 22–23°C. Immediately after death (~30 s), BAT and skeletal muscle temperatures were measured with a digital temperature probe of ~2 mm diameter inserted into the relevant tissue after stab incision. The order of measurement of tissue temperature was randomized among animals to limit any influences of timing on these readings. Plasma and tissues were then removed from 9 control and 18 diet-fed animals and were treated as described above. Interscapular BAT was rapidly excised from the final group of animals (8 control, 8 diet fed), cleaned of any contaminating muscle and fat, and placed in ice-cold 0.9% saline for the guanosine diphosphate (GDP)-binding assay described below.

Commercially available kits were used to measure plasma insulin (Pharmacia Upjohn Diagnostics, Milton Keynes, Bucks, UK), leptin (Linco, Biogenesis, Poole, Dorset, UK), triglycerides (Sigma-Aldrich, Poole, Dorset, UK), glucose (Boehringer Mannheim, Lewes, Sussex, UK), and free fatty acid (Sigma) concentrations. Free fatty acid and triglyceride concentrations were determined in gastrocnemius muscle after extraction from 50–60 mg of tissue by brief sonication in 0.1 M PBS/0.1% BSA (Sigma), followed by centrifugation in an Eppendorf bench-top centrifuge at 10,000 g for 5 min at room temperature. The resulting supernatant was then analyzed with the kits described above.

**Northern Blotting**

RNA was extracted from ~500 mg of epididymal WAT, BAT, and gastrocnemius muscle by disruption in a polytron for 30 s in TRizol reagent (Gibco BRL, Paisley, UK). Twenty-microgram aliquots of total RNA were subjected to electrophoresis on a 1% agarose gel containing formaldehyde and were transferred overnight by capillary blotting to nylon membranes (Boehringer Mannheim). RNA was cross-linked to the membrane by ultraviolet radiation. The nylon membranes were probed with a 3'-labeled digoxigenin 32-mer oligonucleotide (25 ng/ml) against UCP1 or random-labeled digoxigenin cDNA probes against UCP2 and UCP3 in Easyhyb (Boehringer Mannheim) overnight at 42°C, as described previously (30).
UCP blots were visualized by means of an antidigoxigenin antibody (Boehringer Mannheim) conjugated to alkaline phosphatase and 0.2 mM chemiluminescence substrate (Tropix, Bedford, MA), and UCP mRNA band densities were quantitated by computer densitometry (AIS System, Imaging Technology, Brock University, St. Catherine's, ON, Canada). To standardize RNA loading concentrations, probes were stripped off the membrane at 68°C, and the membranes were reprobed against ribosomal 18S with an end-labeled digoxigenin-labeled oligonucleotide probe. Tissue levels of each UCP mRNA were expressed as the ratio of the densities of UCP mRNA/18S rRNA.

**Mitochondrial GDP Binding**

Mitochondria were isolated from BAT samples stored in ice-cold saline from eight controls and eight animals previously fed the palatable diet for 8 wk. The tissues were blotted dry, minced in 8 ml of 0.25 M sucrose buffer (pH 7.2), and homogenized in a glass-Teflon homogenizer. After 10-min centrifugation at 3,500 g and 4°C, the supernatant was filtered through two layers of surgical gauze and spun for an additional 15 min at 10,000 g. The mitochondrial pellets were then resuspended in 1 ml of sucrose buffer and stored at 0°C before use (within 4 h of preparation). Mitochondrial protein content was determined by the Lowry method, with BSA (Sigma) as standard.

Binding of [3H]GDP (Amersham Life Science Products, Buckinghamshire, UK) was determined by use of conditions optimized for isolated mitochondria. Previously, GDP binding was shown to increase in a time-dependent manner and began to plateau after ~3 min (17); utilizing a 7-min incubation, therefore, allowed measurement of near-maximal binding, and values obtained in control animals were similar to those reported for hamster BAT (17). Binding was determined at 22°C by an incubation in buffer containing 100 mM sucrose (BDH Laboratory Supplies, Poole, Dorset, UK), 20 mM TES, K⁺ salt (Sigma), 10 mM choline chloride (Sigma), 5 μM rotenone (Sigma), and 0.1 μCi/ml [3H]GDP (Amersham), pH 7.1. EDTA, K⁺ salt (1 mM), was present in all incubations to inhibit adenylate cyclase activity. Binding was carried out in the presence of two concentrations of unlabeled GDP (Sigma), one within the normal substrate range (2 μM) and one past the point of substrate saturation (200 μM). After incubation for 7 min, bound GDP was separated from free by centrifugation at 10,000 g for 3 min. The unbound radioactivity was then decanted off, and the bound [3H]GDP was assessed by counting in 4 ml of scintillant. Association of GDP with the mitochondria was calculated after allowing for the extramitochondrial space as estimated from [14C]sucrose with the mitochondria was calculated after allowing for was assessed by counting in 4 ml of scintillant. Association of incubations to inhibit adenylate cyclase activity. Binding was shown to increase in a time-dependent manner and began to plateau after ~3 min (17); utilizing a 7-min incubation, therefore, allowed measurement of near-maximal binding, and values obtained in control animals were similar to those reported for hamster BAT (17). Binding was determined at 22°C by an incubation in buffer containing 100 mM sucrose (BDH Laboratory Supplies, Poole, Dorset, UK), 20 mM TES, K⁺ salt (Sigma), 10 mM choline chloride (Sigma), 5 μM rotenone (Sigma), and 0.1 μCi/ml [3H]GDP (Amersham), pH 7.1. EDTA, K⁺ salt (1 mM), was present in all incubations to inhibit adenylate cyclase activity. Binding was carried out in the presence of two concentrations of unlabeled GDP (Sigma), one within the normal substrate range (2 μM) and one past the point of substrate saturation (200 μM). After incubation for 7 min, bound GDP was separated from free by centrifugation at 10,000 g for 3 min. The unbound radioactivity was then decanted off, and the bound [3H]GDP was assessed by counting in 4 ml of scintillant. Association of GDP with the mitochondria was calculated after allowing for the extramitochondrial space as estimated from [14C]sucrose binding (17).

**Statistical Analyses**

Differences in body weight, tissue masses, plasma insulin, leptin, glucose, free fatty acid and triglyceride concentrations, and GDP binding capacity, and those in the levels of each UCP mRNA in control and diet-fed animals, were analyzed by one-way ANOVA followed by Bonferroni-modified t-tests for multiple comparisons.

**RESULTS**

**Two Weeks’ Diet Feeding**

**Metabolic data.** Rats fed the highly palatable diet for 2 wk did not exhibit significant increases above chow-fed controls in body weight (248 ± 4 vs. 229 ± 3 g; P > 0.3) or fat-pad masses, or in plasma insulin, glucose, triglyceride, or free fatty acid concentrations (Table 1). However, these diet-fed animals had significantly higher plasma leptin levels and greater BAT masses and elevated body temperatures (Table 1).

**UCP expression.** After 2 wk of palatable diet feeding, UCP3 mRNA concentrations in gastrocnemius muscle were 126% higher in diet-fed animals (P < 0.05) than in controls (Fig. 1). UCP2 mRNA concentrations in epididymal fat were also elevated by 65% (P < 0.05) (Fig. 2). By contrast, UCP1, UCP2, and UCP3 mRNA levels in BAT were not significantly different at this time (Figs. 3 and 4).

**Eight Weeks’ Diet Feeding**

**Metabolic data.** Eight weeks of palatable-diet feeding resulted in a highly variable degree of obesity, with weight gain ranging from ~300 g, similar to that in chow-fed controls, to almost 500 g. Accordingly, we subdivided the diet-fed rats into “low weight gain” (313–410 g, n = 10) and “high weight gain” groups (427–490 g, n = 8) depending on whether or not body weight gain exceeded the maximum of chow-fed controls (306–410 g) (Table 2). These numbers were consistent with our previous observations that ~50% of diet-fed Wistar rats become high weight gainers (8). A similar bi-modal weight gain pattern has also been reported in Sprague-Dawley rats (13). Energy intake in both high and low weight gain groups was significantly greater than in control rats, but high weight gainers consumed significantly more calories than low weight gainers (Table 2), and there was a highly significant correlation between food intake and weight gain (r² = 0.72, P = 0.004). This could not be attributed to individual dominance affecting feeding behavior, because all the rats in some cages were high weight gainers, whereas in others, all were low weight gainers.

Despite the lack of any significant increase in body weight above controls in low weight gaining animals,
all palatable diet-fed rats were hyperleptinemic and hyperinsulinemic and had significantly raised fat pad and BAT masses, plasma triglyceride concentrations, and gastrocnemius muscle free fatty acid and triglyceride concentrations, compared with controls (Table 2). With the exceptions of BAT mass and muscle free fatty acid and triglyceride concentrations, each of these measures was significantly greater in the high weight gain group. Gastrocnemius muscle mass, an index of the lean tissue mass, was, however, not significantly different between the two obese groups and controls (Table 2). The ratio of plasma glucose to insulin concentrations, an index of insulin sensitivity, suggested that only the high weight gainers had significantly reduced insulin sensitivity, whereas the low weight gainers did not differ significantly from controls (Table 2).

After 8 wk of palatable diet feeding, core body temperature was higher than in chow-fed controls, but this did not differ significantly between low weight and high weight gain groups (Table 2). Similarly, BAT temperature immediately after death was significantly higher in all diet-fed animals than in controls, with no significant difference between the two obese groups (Table 2). By contrast, gastrocnemius muscle temperature was not significantly altered in either low or high weight gain groups compared with controls (Table 2).

**GDP binding.** GDP binding to mitochondria isolated from BAT of animals fed the palatable diet for 8 wk was significantly higher than in control animals, with an approximately twofold increase in binding observed (504 ± 29 vs. 264 ± 15 pmol/mg protein, P < 0.001).

**UCP Expression**

After 8 wk of diet feeding, muscle UCP3 concentrations were again significantly elevated in both low weight gain (127% above controls; P < 0.01) and high weight gain groups (97% above controls; P < 0.01), with no significant difference between the two diet-fed groups (Fig. 1). UCP2 mRNA concentrations in gastrocnemius muscle were raised by 82 (P < 0.01) and 64% (P < 0.05) above controls in low and high weight gainers, respectively (Fig. 5), again with no significant difference between the two groups. By contrast, UCP mRNA concentrations in epididymal fat (Fig. 2) and BAT (Figs. 3 and 4) did not differ significantly from chow-fed controls at 8 wk.

**DISCUSSION**

We have confirmed that inbred Wistar rats have a variable individual feeding response to a highly palatable diet and that this response is associated with widespread metabolic changes, including increased expression of UCP genes.
able diet and develop obesity that ranges from moderate to severe and is significantly correlated with energy intake. Previous studies performed on genetically distinct strains of mice concluded that such differences in susceptibility to the development of obesity were independent of food intake and instead appeared to be related to expression levels of the UCPs and to variations in energy expenditure (20). Outbred Sprague-Dawley rats demonstrate a similar pattern of weight gain. This, too, was reported to be independent of food intake but related to differences in organ sympathetic activity as determined by norepinephrine turnover rates (13, 14). By contrast, our present findings indicate that the variation in adiposity observed in Wistar rats from a genetically homogeneous background is attributable mainly to hyperphagia, with a striking correlation between food intake and body weight gain observed.

Rothwell and Stock (20) suggested that animals overeating palatable food mount an adaptive response to limit excess energy storage. Consistent with this, rats exposed to the highly palatable diet mounted a thermogenic response, as evidenced by increased core temperature, which may have originated in the hypertrophic BAT. This adaptive thermogenic mechanism is thought to account for the greater capacity of young rats to resist the onset of obesity (15) and probably explains the failure of animals fed a palatable diet for 2 wk to gain significant body weight or fat mass compared with chow-fed controls. However, the magnitude of this thermogenic effect was unrelated to weight or fat gain during 8 wk of diet feeding. Previous studies using Sprague-Dawley rats also reported that a similar activation of thermogenesis failed to prevent the development of dietary-induced obesity (15). We therefore conclude that, at least in the strain and age of rat used in this study, the degree of adiposity is not determined by energy disposal but is related primarily to differences in food intake.

We were not able to measure whole body energy expenditure directly in this study, and our recordings of core body temperature and tissue temperatures immediately after death provide only an indirect picture of events. Certain physical factors that may influence these measurements could vary systematically between experimental groups, e.g., the amount of insulating body fat that could affect heat loss during life.
proton conductance pathway in the absence of elevated fed rats (2). This measured increase in activity of the animals, consistent with previous studies in cafeteria-uncoupling activity, was also increased in diet-fed thermogenic capacity of BAT. GDP binding, a measure of BAT hypertrophy, and therefore the total significantly different from controls, but the total amount of UCP1 mRNA (relative to 18S rRNA) were not sign-
ificantly different from controls. In addition, it was ensured that consistent environmental and experimental conditions were main-
tained. Therefore, comparison of these values between the different groups of animals is probably valid.

We examined UCP mRNA in BAT, WAT, and skeletal muscle, the most abundant sites of UCP expression and those commonly studied, in an attempt to determine their possible involvement in the development of adiposity. Several other depots of UCP (e.g., cardiac tissue and liver) were not studied, and we acknowledge that the activity of these additional sites of expression may influence our current findings.

Both low and high weight gainers exhibited marked BAT hypertrophy with significantly elevated BAT temperature, suggesting that this tissue, rather than muscle, is the source of the raised core temperature. Levels of UCP1 mRNA (relative to 18S rRNA) were not significantly different from controls, but the total amount of UCP1 would be increased in diet-fed rats as a consequence of BAT hypertrophy, and therefore the total thermogenic capacity of BAT. GDP binding, a measure of uncoupling activity, was also increased in diet-fed animals, consistent with previous studies in catareria-fed rats (2). This measured increase in activity of the proton conductance pathway in the absence of elevated

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Table 2. Changes in body weight, tissue mass, and blood and muscle analytes in low and high weight gain groups fed the highly palatable diet for 8 wk vs. chow-fed controls

<table>
<thead>
<tr>
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<th>Low Weight Gain</th>
<th>High Weight Gain</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>169 ± 7</td>
<td>164 ± 6</td>
</tr>
<tr>
<td>Final</td>
<td>507 ± 10</td>
<td>508 ± 8</td>
</tr>
<tr>
<td>Fat pad mass, g</td>
<td>2.8 ± 0.1</td>
<td>2.7 ± 0.0</td>
</tr>
<tr>
<td>Epididymal mass, g</td>
<td>5.3 ± 0.3</td>
<td>9.0 ± 0.3‡</td>
</tr>
<tr>
<td>Perirenal mass, g</td>
<td>3.0 ± 0.2</td>
<td>7.1 ± 0.3‡</td>
</tr>
<tr>
<td>BAT mass, g</td>
<td>0.58 ± 0.0</td>
<td>0.84 ± 0.1†</td>
</tr>
<tr>
<td>Gastrocnemius mass, g</td>
<td>22.7 ± 1.3</td>
<td>30.0 ± 1.0†</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>36.2 ± 0.2</td>
<td>37.0 ± 0.2†</td>
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<tr>
<td>Postmortem muscle</td>
<td>35.4 ± 0.2</td>
<td>35.6 ± 0.2</td>
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<tr>
<td>temperature, °C</td>
<td>4.7 ± 0.2</td>
<td>5.9 ± 0.22†</td>
</tr>
<tr>
<td>Plasma leptin, ng/ml</td>
<td>22.7 ± 1.3</td>
<td>30.0 ± 1.0†</td>
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<tr>
<td>Plasma insulin, μU/ml</td>
<td>2.0 ± 0.0</td>
<td>2.6 ± 0.1†</td>
</tr>
<tr>
<td>Insulin/glucose ratio</td>
<td>0.54 ± 0.1</td>
<td>0.48 ± 0.0</td>
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<tr>
<td>Muscle triglyceride, mM</td>
<td>22.5 ± 0.8</td>
<td>31.1 ± 1.7†</td>
</tr>
<tr>
<td>Muscle free fatty acid, mmol/g</td>
<td>0.6 ± 0.0</td>
<td>1.7 ± 0.1†</td>
</tr>
<tr>
<td>Energy intake, KJ/day</td>
<td>413 ± 11</td>
<td>455 ± 44*</td>
</tr>
<tr>
<td>Controls (n = 9)</td>
<td></td>
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<tr>
<td>Low Weight Gain (n = 10)</td>
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<td>High Weight Gain (n = 8)</td>
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Data shown as means ± SE. *P < 0.05, †P < 0.01 vs. controls; ‡P < 0.05, §§P < 0.01 vs. low-weight gainers.

UCP1 mRNA levels suggests that posttranscriptional changes (e.g., improved stability of UCP1 mRNA) may occur to increase the translation of functional UCP1. Such changes are not reflected by mRNA measurements. Although combining measurements of UCP mRNA and protein content would be more informative, the latter was found not to be possible utilizing the antibodies commercially available at present. Alternatively, the observed increase in BAT GDP binding could reflect unmasking of existing binding sites rather than an actual increase in the amount of binding protein (10).

The BAT UCP1 results conflict with previously published studies in which elevated UCP1 mRNA levels were observed in dietary-obese animals (22). Age has been shown to reduce diet-induced thermogenic activity and increases in UCP content of BAT, with both being virtually absent in 6.5-mo-old rats (21). Yet this is unlikely to offer a suitable explanation, because our animals were only 4 mo old at the end of the study. Diet composition can also influence UCP mRNA levels; for example, enrichment of a high-fat diet with n-3 polyunsaturated fatty acids has been shown to stimulate BAT thermogenic activity (assessed by GDP binding) without altering UCP content (18), whereas increased palatability of the diet per se also stimulates BAT thermogenic activity (12). The composition of our diet

![Fig. 5. UCP2 mRNA levels in gastrocnemius muscle of animals fed the highly palatable diet for 2 or 8 wk vs. chow-fed controls. Data shown are means ± SE for groups of 8 or 9 rats, with all values normalized to the mean of the control values. *P < 0.05, **P < 0.01 vs. chow-fed controls.](http://ajpendo.physiology.org/DownloadedFrom/10.2307/3886387)
may therefore contribute to the divergence from previously published results.

UCP2 mRNA expression in WAT has been shown to be regulated by dietary fat consumption and by the genetic background of mice (25). In the present study, elevated UCP2 mRNA concentrations were observed in WAT of all animals exposed to the palatable diet for 2 wk, but this increase was not maintained in either low or high weight gain groups after 8 wk of diet feeding. This suggests that, in rats, the expression of UCP2 is determined by duration of the energy challenge. Similar observations have also been made for mice fed a high-fat diet, with UCP2 mRNA levels reported to be increased twofold in WAT of obesity-resistant mice fed a high-fat diet for 2 wk (25) but to be unchanged in animals of the same genetic background fed the diet for 8 wk (7). This response may represent an early attempt either to prevent the development of obesity by increasing thermogenesis in WAT or perhaps to limit lipid accumulation in adipocytes, with these roles being assumed by other UCP depots with continuing dietary consumption. Its contribution to the overall degree of adiposity is difficult to assess, because diet-fed animals could not be stratified into high or low weight gainers at this early stage.

The dietary-obese mice reported by Surwit et al. (25) showed no detectable changes in the expression of either UCP2 or UCP3 in gastrocnemius or soleus muscle. By contrast, all our diet-fed rats showed increased UCP2 concentrations in gastrocnemius muscle, but only after prolonged exposure (8 wk) to the diet and irrespective of weight gain. This suggests that UCP2 in muscle is recruited only when signals, possibly raised free fatty acid levels (29), reach a critical level. The pattern of change differs from that observed for UCP2 expression in WAT, consistent with the observation of tissue-specific differences in the mechanisms regulating UCP2 expression in mice (25).

Elevated UCP3 mRNA concentrations were also observed in gastrocnemius muscle of diet-fed rats after 2 and 8 wk, indicating that signals mediating the altered UCP3 gene expression reach a maximal level early after dietary challenge. The unchanged BAT UCP3 mRNA levels after palatable diet feeding further highlight the marked tissue- and species-specific differences in the regulation of UCP expression (25).

Although UCP mRNA expression was elevated to comparable degrees in gastrocnemius muscle of both low and high weight gain groups, it seems unlikely that enhanced thermogenesis in muscle is responsible for the increased core temperature, because muscle temperature was not significantly raised in either dietary obese group, in contrast to the clear rise in BAT temperature measured in an identical fashion. However, we acknowledge that differences in heat production could be masked postmortem. The suggestion that muscle UCPs mediate increased energy disposal is controversial. UCP2 and UCP3 expression is increased in fasted rats, which show decreased energy expenditure (29), arguing against their involvement in classical thermogenic mechanisms. Upregulation of UCP3 in skeletal muscle also occurs in exercise-trained mice (26). In starvation and physical exercise, stored fat mobilized as fatty acid utilization by the muscle is increased, suggesting a role for UCPs in fuel utilization. Elevated free fatty acid levels after Intralipid and heparin infusion produce UCP3 mRNA levels comparable to those observed during fasting (29), providing further evidence that elevated free fatty acids are important signals for muscle UCP induction. Overconsumption of a palatable diet also results in elevated levels of circulating lipids, leading to raised muscle levels of free fatty acid and triglycerides in the dietary-obese animals. As obesity results in the accumulation of fat not only in adipocytes but also in other tissues, including skeletal muscle (33), pancreatic islet β-cells, and other organs (23), elevated muscle UCP levels in the dietary-obese rats may therefore reflect the increased utilization of lipids as an energy source in an attempt to limit their accumulation in muscle.

Leptin is another potential regulator of UCP expression. Elevated plasma leptin levels may increase UCP2 (32) and UCP3 (6) expression in adipose tissue and muscle of fed animals. Additionally, leptin has been shown to deplete the fat content of adipocytes and nonadipocytes by preventing the conversion of free fatty acid to triglyceride and by promoting free fatty acid oxidation (23). Altered leptin levels could therefore represent the initial trigger for the free fatty acid regulation of UCP induction. Yet fasting increases UCP levels despite a significant reduction in plasma leptin (29), and exogenous leptin did not prevent the induction of UCP3 by fasting (29) or alter UCP2 expression in A/J or B6 mice (25). Here, we found markedly increased plasma leptin levels in all diet-fed animals, with significantly higher levels in the high weight gainers than in the low weight gainers. Muscle UCP2 and UCP3 expression was increased to approximately the same degree in both obese groups, arguing against a regulatory role of leptin, although the “leptin resistance” associated with dietary obesity (27) could modulate the relationship between plasma leptin levels and UCP expression. Although such dietary-obese animals exhibit peripheral leptin resistance (27), they have been shown to retain sensitivity to centrally administered leptin (27). A primary role of leptin in metabolic homeostasis is to provide information on the amount of body fat through binding to leptin receptors within the central nervous system and thereby modulating functions that regulate food intake and energy expenditure, such as sympathetic nerve activity in BAT (8). Plasma leptin levels may not provide a true reflection of these central leptin concentrations (3), which may be responsible for regulating UCP expression.

In conclusion, unselected adult Wistar rats fed a highly palatable diet over 8 wk demonstrated variable hyperphagia that was proportional to weight gain. All diet-fed animals showed elevated body temperature, BAT hypertrophy, and increased UCP2 and UCP3 expression in muscle, but these changes were unrelated to the severity of obesity. We conclude that energy
intake is the most important individual determinant of adiposity in unselected Wistar rats.

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